Supporting Information 4

Validation of Three Methods: Original Pyrogallol Method, Improved Pyrogallol Method and NBT Method

XICAN LI

School of Chinese Herbal Medicine, Guangzhou University of Chinese

Medicine, Guangzhou, China

1 The **precision** of improved pyrogallol method, original pyrogallol method and

NBT method

Tab. V1　Improved pyrogallol method for Trolox (pH7.4, λ=325nm, 37□, ΔA$_0$ = 0.592)

Trolox concentration　μg/mL	ΔA	Inhibition %	Mean	RSD%
	0.537	9.341029		
125	0.54	8.834558	9.23	3.80
	0.536	9.509853		
	0.475	19.80811		
250	0.476	19.63929	19.86	1.30
	0.473	20.14576		
	0.35	40.91111		
500	0.346	41.5864	40.79	2.08
	0.356	39.89817		
	0.213	64.03999		
750	0.22	62.85822	62.69	2.30
	0.23	61.16998		
	0.13	78.05238		
1000	0.125	78.8965	78.78	0.87
	0.122	79.40297		
	0.058	90.2077		
1250	0.054	90.883	90.32	0.57
	0.06	89.87006		

Tab. V2 Original pyrogallol method for Trolox (pH8.2, λ=420nm, R.T., ΔA_0 = 0.596)

Trolox concentration μg/mL	ΔA	Inhibition %	Mean	RSD%
250	0.553	7.266578	8.16	12.55
	0.549	7.937344		
	0.541	9.278876		
750	0.498	16.48961	17.38	4.85
	0.492	17.49576		
	0.488	18.16653		
1250	0.449	24.7065	25.10	3.91
	0.451	24.37111		
	0.44	26.21572		
1750	0.398	33.25877	34.04	2.22
	0.393	34.09722		
	0.389	34.76799		
2250	0.33	44.66179	44.55	3.02
	0.323	45.83563		
	0.339	43.15257		
2500	0.291	51.20176	51.43	0.75
	0.291	51.20176		
	0.287	51.87253		

Tab. V3   NBT assay for Trolox (pH7.4, R.T., $A_{0, 560nm}$ = 1.236)

Trolox concentration µg/mL	A_{560nm}	Inhibition %	Mean	RSD%
125	1.133	8.333333	6.63	64.31
	1.115	9.789644		
	1.214	1.779935		
250	1.057	14.4822	18.82	35.57
	1.045	15.45307		
	0.908	26.53722		
500	0.712	42.39482	41.64	5.29
	0.752	39.15858		
	0.7	43.3657		
750	0.417	66.26214	62.59	5.31
	0.497	59.78964		
	0.473	61.73139		
1000	0.465	62.37864	71.30	10.86
	0.305	75.32362		
	0.294	76.21359		
1250	0.245	80.17799	80.45	1.54
	0.225	81.79612		
	0.255	79.36893		

2 The **linearity** of improved pyrogallol assay

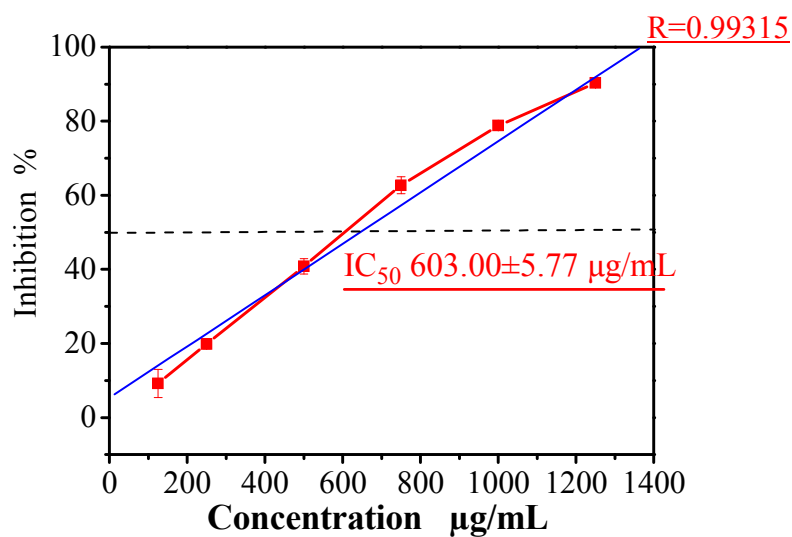Note: The data below with <u>underline</u> were mentioned in the main text (Results and discussion).

Fig. V1   Linearity graph of improved pyrogallol assay for Trolox
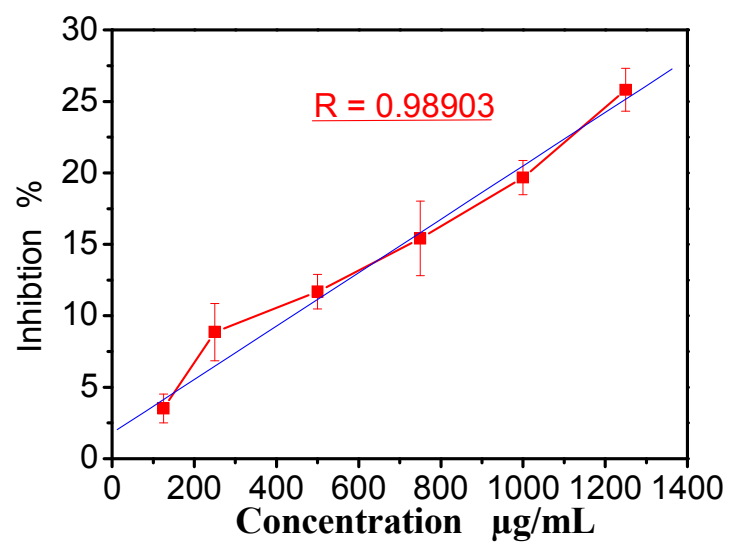Data were presented as the mean ± SD for three replicates.

Fig. V2   Linearity graph of original pyrogallol assay for Trolox
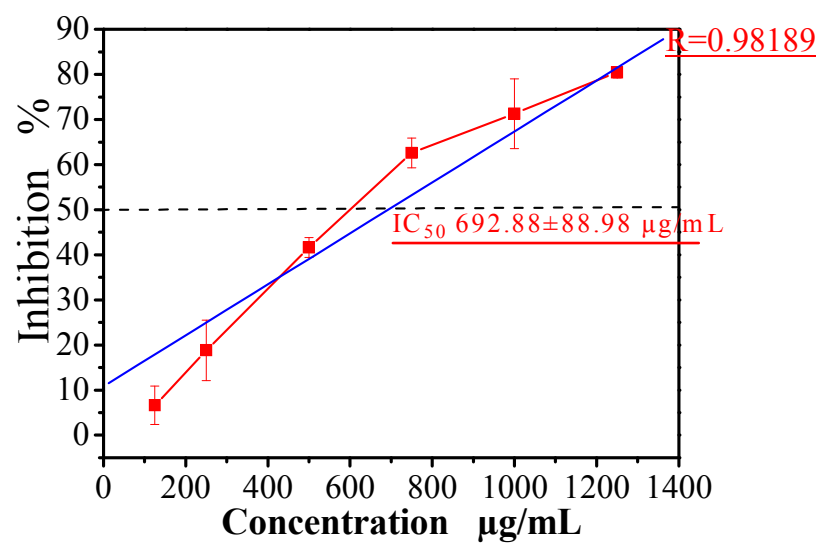Data were presented as the mean ± SD for three replicates.

Fig. V3   Linearity graph of NBT method for Trolox
Data were presented as the mean ± SD for three replicates.

3   The **reproducibility** of improved pyrogallol method, original pyrogallol

method and NBT method

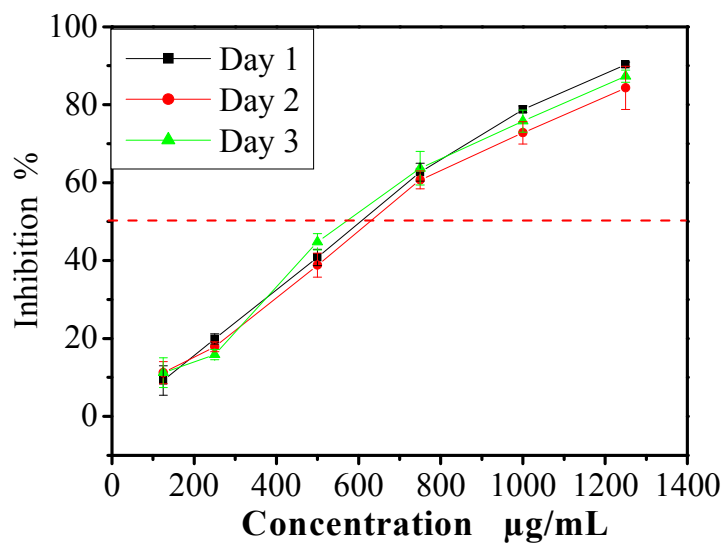Note: The data below with <u>underline</u> were mentioned in the main text (Results and discussion).

Fig. V4   The reproducibility of improved pyrogallol method for Trolox (3 days)
Data were presented as the mean ± SD for three replicates.

According to the Figure, three IC_{50} values was calculated as: 603.00,681.07, 648.93,μg/mL for day 1, day 2 and day 3. The RSD % of three IC_{50} values were <u>6.09%</u>.
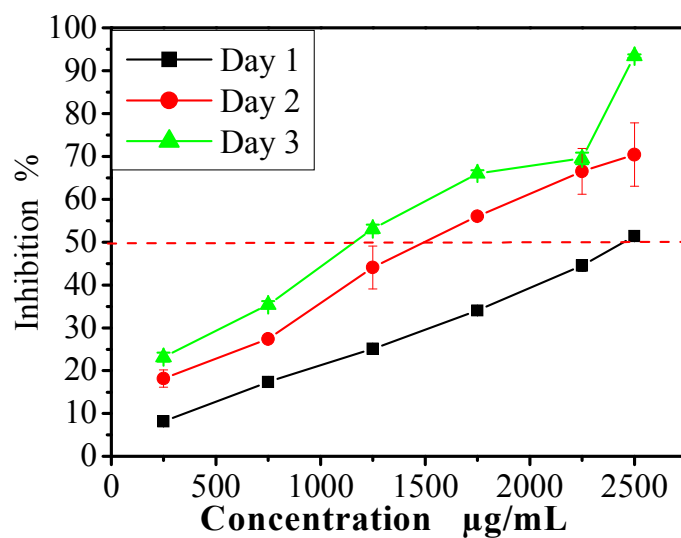
Fig. V5 The reproducibility of original pyrogallol method for Trolox (3 days)
Data were presented as the mean ± SD for three replicates.

According to the Figure, three IC_{50} values was calculated as: 2416.67, 1578.79, 1218.15μg/mL for day 1, day 2 and day 3. The RSD % of three IC_{50} values were 35.38%.
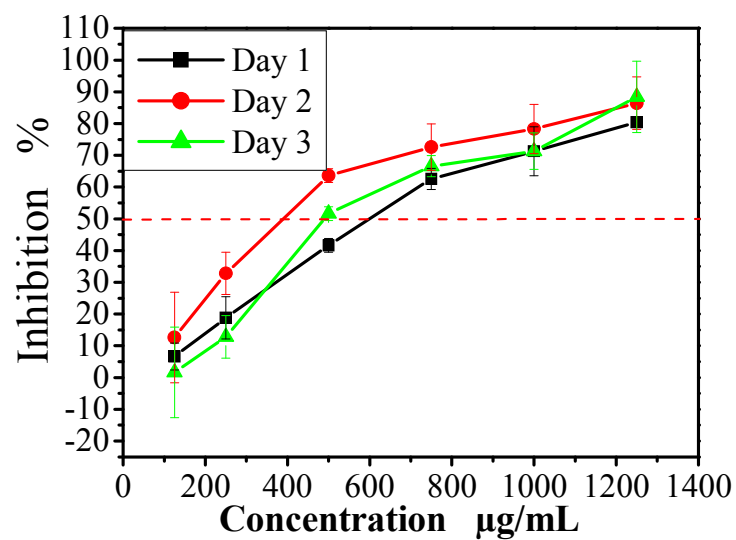
Fig. V6   The reproducibility of NBT method for Trolox (3 days)
Data were presented as the mean ± SD for three replicates.

According to the Figure, three IC_{50} values was calculated as: 692.88, 520.37, 662.39 µg/mL for day 1, day 2 and day 3. The RSD % of three IC_{50} values were 14.72%.